IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402, p- ISSN: 2319-2399.Volume 20, Issue 5 Ser. 1 (May 2026), PP 15-18
www.iosrjournals.org

Modulatory Effects of Spices Mixture on Oxidative Stress of Albino Rats Exposed to Calcium Carbide Fume

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Abstract
This study evaluated the modulatory effects of a spice mixture on immune function, inflammation, reproductive hormones, and oxidative stress in albino rats exposed to calcium carbide (CaC) fumes. Thirty-six (36) male Wistar albino rats were randomly assigned into six groups (n = 6). The normal control received feed and water only, while other groups were exposed to CaC fumes for 8 hours daily. The positive control was exposed but untreated. Standard control groups received vitamin C (100 mg/kg) either 8 hours before or after exposure, whereas prophylactic and treatment groups received a spice mixture (300 mg/kg) before or after exposure, respectively. Oxidative stress was evident in exposed rats, with elevated catalase (0.58 ± 0.02 U/L) and malondialdehyde (0.66 ± 0.02 mg/dL), while spice treatment improved antioxidant status (CAT: 0.40 ± 0.01 U/L; MDA: 0.27 ± 0.02 mg/dL). Overall, prophylactic and therapeutic treatment with the spice mixture and vitamin C attenuated calcium carbide–induced oxidative stress. These findings suggest that the spice mixture possesses significant antioxidant properties against CaC fume toxicity.

Date of Submission: 24-04-2026 Date of Acceptance: 04-05-2026

I. Introduction

Calcium carbide (CaC_2), commonly known as carbide or calcium acetylide, is mostly used to produce acetylene and calcium cyanamide. CaC_2 of technical grade is grey or brown and consists of about 85 % of CaC_2, with other compounds including silicon carbide, calcium phosphide, calcium oxide, calcium nitride, and calcium sulphide (Danish *et al.*, 2015). However, in pure form, CaC_2 is colourless and decomposes in water, forming flammable acetylene gas and calcium hydroxide ($Ca(OH)_2$) (Okeke *et al.*, 2022).

The potent antioxidant and anti-inflammatory agent could modulate CaC_2 toxicity. Garlic possesses immunomodulatory properties that are facilitated by its ability to regulate cytokine production and enhance the activity of immune cells, leading to the release of antibodies and the initiation of an immune response (Ejiogu, 2024). Ginger possesses anti-inflammatory properties, anti-thrombotic properties, cholesterol-lowering properties, blood pressure-lowering properties, anti-microbial properties, anti-oxidant properties, anti-tumour properties, and hypoglycaemic properties (Shahrajabian *et al.*, 2019). Despite the reported individual medicinal or therapeutic potential, there is paucity of literature on the combinatory effects of ginger and garlic on immunity, inflammation, reproduction and oxidative stress of albino rats exposed to calcium carbide fume.

The illegal use of CaC_2 in fruit ripening remains prevalent in many developing countries, including Nigeria, despite regulatory warnings (FAO, 2019). Upon exposure to moisture, calcium carbide releases acetylene gas, a ripening agent that mimics ethylene but may contain toxic impurities such as arsenic and phosphorus hydride (Onwuka & Chamberlain, 2020). Inhalation or ingestion of CaC_2 residues has been linked to oxidative stress, immune dysfunction, hormonal imbalances, inflammation and reproductive toxicity (Oyeleke et al., 2018). However, public awareness and intervention strategies remain limited.

Natural spices like turmeric, ginger, garlic and cloves are rich in bioactive compounds with antioxidant, anti-inflammatory, and immunomodulatory properties (Aggarwal *et al.*, 2013). These spices have shown protective effects against chemical-induced toxicity in experimental models. Nevertheless, there is limited scientific data on the collective (synergistic) modulatory effects of a spice mixture in mitigating the toxicological impact of CaC_2 exposure. Given the increasing health risks associated with consumption and handling of carbide-ripened produce, it is essential to investigate the potential of natural dietary interventions.

This study therefore seeks to explore the protective effects of a spice mixture on immune function, inflammation, reproductive health, and oxidative stress in albino rats exposed to CaC_2 fume, thus providing scientific evidence for safer food practices and possible therapeutic option. This study seeks to evaluate the modulatory effects of spices mixture on immunity, inflammation, reproduction and oxidative stress of albino rats exposed to calcium carbide fume.

II. Materials And Methods

Biological materials

These include male Wistar rat, rat feed, spices mixture, vitamin C, distilled water, and blood samples.

Chemical materials

Analytical grade calcium carbide (CaC_2) was obtained from Riedel-De HaenAGSeelze-Hannover, Germany. The reagents used were commercial kits purchased from Randox Laboratories (UK).

Methods

Preparation of spice extract

Fresh garlic and ginger were obtained and washed properly with water, the sample was separately peeled, chopped into small pieces, and blended into a fine paste using an electrical blender. Then 250g each of the ground garlic and ginger was weighed using an analytical weighing balance and was soaked together in a 2.5litre(2500ml) of absolute ethanol for 72hours. The mixture was filtered using a muslin cloth to remove solid residues. The filtrate was left to evaporate at room temperature until a semi-solid concentrate was obtained. The final extract was stored in an airtight container until use.

Preparation of Vitamin C

Vitamin C tablets were obtained from a reputable pharmacy in Abakaliki, Ebonyi State Nigeria. Exactly 2g (5 tablets) of vitamin C tablets was dissolved in 100ml of distilled water to obtain stock solution of 0.02g/ml.

Experimental design

Sixty (60) male Wistar albino rats were randomly assigned into six groups, 10 in each group. Normal control group (NOC) received feed and water only while other groups were exposed to calcium carbide fumes for eight (8) hours daily. Positive control (POC) was left untreated after exposed to calcium carbide fumes, while the standard controls (SDC_1 and SDC_2) were given 100 mg/kg of vitamin C 8 hours before exposure to CaC_2 fume for (SDC_1), and 8 hours after exposure to CaC_2 fume for (SDC_2). However, the prophylactic (PLG) and the treatment (TRG) groups received 300 mg/kg spices (Ugwuja *et al*., 2016) mixture 8 hours before exposure to calcium carbide fume, and 8 hours after exposure to calcium carbide fume, respectively. All animals were given water *ad libitum*. The dose of vitamin C used in the present study was in accordance with published works (Obafemi *et al*., 2019). All administration except CaC_2 will be through oral route for forty-two (42) days.

Blood samples was collected through ocular puncture with the animals under diethyl ether anastasia. Blood samples was collected using lithium heparin bottles after which samples were centrifuged at 2000g for five minutes. The plasma was dispensed into plain bottles and stored refrigerated until analyses was carried out on them.

Laboratory Analysis

Determination of Oxidative Stress Markers (Catalase, SOD And MDA):

Catalase activity was examined by monitoring the decomposition of hydrogen peroxide (H2O2) at 240 nm using a spectrophotometer. SOD levels was conducted using double-antibody sandwich ELISA while MDA was examined using thiobarbituric acid (TBA) reaction. This method involves reacting MDA with TBA at high temperatures and acidic conditions, forming a pink-colored adduct that is measured spectrophotometrically at 532 nm (Tekin & Seven, 2022).

III. Result And Discussion

Effect of Spices Mixture (SM) on Oxidative Indices of Rats Exposed to Calcium Carbide Fume

Table 4 shows a significant increase in the serum levels of catalase POC ($P < 0.05$; $P = 0.0001$) and SDC2 ($P < 0.05$; $P = 0.004$) compared to NOC. No significant difference in the levels of serum catalase was observed when SDC1 ($P > 0.05$; $P = 0.764$), PRG ($P > 0.05$; $P = 0.833$) and TRG ($P > 0.05$; $P = 1.000$) were compared to NOC. Furthermore, there was a significant increase in the serum levels of catalase in SDC1 ($P < 0.05$; $P = 0.0001$), SDC2 ($P < 0.05$; $P = 0.0001$), PRG ($P < 0.05$; $P = 0.0001$) and TRG ($P < 0.05$; $P = 0.0001$) compared to POC. SOD shows a significant increase in POC ($P < 0.05$; $P = 0.0001$), SDC1 ($P < 0.05$; P =

0.001), SDC2 ($P < 0.05$; P = 0.003), PRG ($P < 0.05$; P = 0.0001) and TRG ($P < 0.05$; P = 0.0001) compared to NOC. A significant decrease was observed in SDC1 ($P < 0.05$; P = 0.0001), SDC2 ($P < 0.05$; P = 0.0001), PRG ($P < 0.05$; P = 0.0001) and TRG ($P < 0.05$; P = 0.012) compared to POC. While in MDA, the study showed a significant increase and decrease in POC ($P < 0.05$; P = 0.0001) and PRG ($P < 0.05$; P = 0.027), respectively, compared to NOC. This study further showed no significant difference in SDC1 ($P > 0.05$; P = 0.929), SDC2 ($P > 0.05$; P = 0.724), and TRG ($P > 0.05$; P = 0.791) compared to NOC. Also observed in this study is a significant decrease in SDC1 ($P < 0.05$; P = 0.0001), SDC2 ($P < 0.05$; P = 0.0001), PRG ($P < 0.05$; P = 0.0001) and TRG ($P < 0.05$; P = 0.0001) compared to POC. This indicates oxidative stress and an adaptive up-regulation of antioxidant enzymes due to fume toxicity.

However, the standard control groups (Vitamin C groups); (SDC1 & SDC2), showed moderate reduction in oxidative stress, normalizing CAT and MDA levels and maintaining SOD near normal values. Prophylactic (PRG) and treatment (TRG) groups that received the spices mixture (SM) before or after exposure showed restoration of antioxidant balance close to the normal control. In particular, MDA levels (0.20-0.27 mg/dl) were low, signifying lipid peroxidation inhibition, while SOD and CAT were maintained within physiological range. Thus, SM showed protective and therapeutic antioxidant potential comparable to vitamin C.

Table 1: Effect of spices mixture (SM) on oxidative indices of rats exposed to calcium carbide fume

Parameters	CAT (U/L)	SOD (U/L)	MDA (mg/dl)
NOC	0.40±0.02	167.50±8.55	0.27±0.02
POC	0.58±0.02^{a}	261.40±6.29^{a}	0.66±0.02^{a}
SDC1	0.40±0.02^{cb}	203.25±8.34^{ab}	0.26±0.02^{cb}
SDC2	0.48±0.02^{ab}	197.50±2.53^{ab}	0.26±0.02^{cb}
PRG	0.39±0.01^{cb}	207.40±4.11^{ab}	0.20±0.01^{ab}
TRG	0.40±0.01^{cb}	238.25±3.75^{ab}	0.27±0.02^{cb}

Values are expressed as mean ± SEM. a indicate significant difference and c indicate no significant difference, when compared to NOC. b indicates significant difference and d indicate no significant difference, when compared to POC. The significance level was set at $P < 0.05$.

NOC: Normal control; **POC:** CaC_2 exposed control; **SDC1:** Standard control 1 (prophylactic treatment with vitamin C); **SDC2:** Standard control 2 (treated with vitamin C after CaC_2 exposure; **PRG:** Prophylactic group (pretreated with spices mixture before CaC_2 exposure); **TRG:** Treatment group (treated with spices mixture after CaC_2 exposure

Table 2: Effect of spices mixture (SM) on plasma oxidative stress indices of rats exposed to calcium carbide fume

Parameters	TNF-α (pg/ml)	IL-1β (pg/ml)	IL-6 (pg/ml)
NOC	23.87±0.50	1.66±0.22	27.20±1.60
POC	53.96±2.93^{a}	6.96±0.39^{a}	66.58±2.56^{a}
SDC1	45.44±2.58^{ab}	4.91±0.26^{ab}	59.81±1.06^{ad}
SDC2	43.90±3.28^{ab}	4.96±0.11^{ab}	60.91±3.36^{ad}
PRG	43.61±2.04^{ab}	4.97±0.12^{ab}	64.84±4.02^{ad}
TRG	22.81±0.72^{cb}	2.42±0.48^{cb}	32.30±1.31^{cb}

Values are expressed as mean ± SEM. a indicate significant difference and c indicate no significant difference, when compared to NOC. b indicates significant difference and d indicate no significant difference, when compared to POC. The significance level was set at $P < 0.05$.

NOC: Normal control; **POC:** CaC_2 exposed control; **SDC1:** Standard control 1 (prophylactic treatment with vitamin C); **SDC2:** Standard control 2 (treated with vitamin C after CaC_2 exposure; **PRG:** Prophylactic group (pretreated with spices mixture before CaC_2 exposure); **TRG:** Treatment group (treated with spices mixture after CaC_2 exposure.

Exposure to calcium carbide (CaC) or carbide-ripened material initiates a chain of redox and inflammatory events that explain the pattern seen in the present study. CaC releases acetylene when it comes in contacts with moisture and, in many industrial/food-grade preparations carried out with CaC , it also carries trace impurities (arsenic, phosphides) that can promote free-radical formation and metal-catalyzed redox cycling; experimental models report organ oxidative stress, GSH perturbation and elevated markers of lipid peroxidation after CaC exposure (Ouma et al., 2022; Bafor *et al*., 2019).

However, the acute oxidative signal from CaC (and its contaminants/metabolites) raises ROS in cells and mitochondria; ROS oxidize polyunsaturated lipids producing malondialdehyde (MDA), which is the biochemical product of lipid peroxidation. The significant increase in MDA observed in the positive control group (POC) therefore indicates robust membrane lipid attack. Cells attempt to counter the ROS burst by upregulating primary enzymatic defenses, including superoxide dismutase (SOD), which converts superoxide anion to hydrogen peroxide. The enzyme catalase (CAT) decomposes hydrogen peroxide to water and oxygen. The significant SOD and CAT increases in the POC animals are consistent with an acute, compensatory induction of these enzymes in response to elevated superoxide and H O   (Ouma et al., 2022). If ROS production overwhelms defenses, however, downstream damage (lipid peroxidation, protein oxidation, DNA oxidation) accelerates despite higher SOD/CAT activity; MDA remains elevated because upregulated enzymes seemed to be insufficient or mislocalized, or because other antioxidant pools (e.g., glutathione, thioredoxin) are depleted or oxidized. This pattern; higher antioxidant enzyme activities together with higher MDA is classically seen when oxidative insult is acute and large, not when antioxidant systems are intact and unchallenged. Against that pathologic backdrop, both vitamin C and the spices mixture (SM) act through partly overlapping but distinct antioxidant routes to restore redox balance. Vitamin C is a high-capacity aqueous phase radical scavenger and can regenerate other antioxidants (e.g., tocopherol), directly quench radical species and limit propagation of lipid peroxidation; it also supports enzyme cofactor functions and stabilizes cellular redox couples (Alberts et al., 2025). Spice phytochemicals a heterogeneous mix of polyphenols, flavonoids, phenolic terpenes and sulfur-containing compounds reduce oxidant burden via three pathways: (a) direct radical scavenging and metal chelation (preventing Fenton chemistry), (b) modulating redox-sensitive transcription factors such as Nuclear factor erythroid 2-related factor 2 (Nrf2) to enhance expression of phase II antioxidant genes, and (c) stabilizing membranes and mitochondrial function (Yashin et al., 2017; Lourenço et al., 2019). Hence, prophylactic and therapeutic SM administration returned SOD and CAT to nearer-physiological levels while lowering MDA (PRG and TRG MDA ≈ NOC). That combination indicates two things. Firstly, SM may have lowered the upstream ROS flux (so inducible SOD/CAT expression did not need to remain elevated), and secondly, SM might have limited ongoing lipid peroxidation either by direct interception of chain-carrying radicals or by chelating redox-active metal contaminants from carbide residues. The nearly comparable protection by SM and vitamin C is plausible because both interventions target ROS directly, but SM brings multiple bioactive compounds that act in parallel on free radical neutralization, metal chelation, and transcriptional induction of cellular defences (Yashin et al., 2017).

Taken together, the analytical data of our findings is coherent and well aligned with current literature: CaC   fume exposure produces an ROS-driven cascade (leukocyte redistribution, cytokine release, endocrine suppression and lipid peroxidation /induction of SOD/CAT), and both vitamin C and spices mixture attenuate these effects by scavenging radicals, chelating pro-oxidant metals, modulating redox-sensitive transcription factors (e.g., Nrf2 and NF-κB), and preserving tissue function. The spices mixture likely confers multi-targeted protection (direct antioxidant, transcriptional and anti-inflammatory) that complements the single-molecule radical scavenging of ascorbate, explaining the comparable or, in some result, superior outcomes observed in the present study.

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